DETAILED ACTION

Applicant's response and amendments filed 10 June 2008 have been received and entered.

Claims 37-57, 68 and 71 have been examined on the merits.

Withdrawn Claim Objections

In view of Applicant's amendment to claim 40, the previous objection to the claim has been withdrawn.

Maintained Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 37-57, 68 and 71 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In claims 37 and 71, Applicant recites the phrase "the difference in intensities of the mean fluorescences of the fluorescent agents is at least double on a logarithmic scale" in lines 6-8 of step (f) and lines 5-7 of step (g), respectively. In particular, it is unclear what Applicant means by "at least double on a logarithmic scale" (i.e., is Applicant referring to a particular logarithmic scale?)

Claim 49 recites the limitation "microparticles" in line 2. There is insufficient antecedent basis for this limitation in the claim. In particular, the parent claim, claim 37,

provides no support for microparticles, and therefore, claim 49, which is drawn to a step wherein "microparticles are further separated from the sample" lacks antecedent basis for microparticles.

All other claims depend directly or indirectly from rejected claims and are, therefore, also rejected under USC 112, second paragraph for the reasons set forth above.

Maintained Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 37-48, 50-53, 55-57, 68 and 71 stand rejected under 35 U.S.C. 102(b) as being anticipated by Schut et al (WO 99/10533).

A method is claimed for identifying and for measuring the portion in a sample one or more microorganisms and/or microorganism species.

Schut et al teach a method of measuring *Bifidobacterium* species in fecal samples by labeling the sample with Cy5-labeled Bif probe, a 16S rRNA probe specific for *Bifidobacterium* species, and also labeling the sample with universal probes, such as FITC-labeled Eub (stains all bacteria), or with general nucleic acid stains such as YOPRO-1, or SYTO 9. Schut et al teach that the samples were then subjected to flow cytometry for fluorescence readings of the FISH procedure to determine cell counts of

the desired species. Additionally, Schut et al teach methods utilizing flow cytometric analysis of samples to study the effect of prebiotics and probiotics on human intestinal microflora, to monitor mixed bacterial populations that are essential for industrial fermentation processes, to rapidly detect microbial contamination in products or production tools, or to identify and quantify the presence and activity of microorganisms in bioremediation processes and wastewater treatment systems (see, for example, page 19, lines 15-36). Schut et al teach that that best separation between fluorescent intensities is achieved with Cy5-labeled Bif probe and YOPRO-1 at 10x diluted commercial concentration, which is demonstrated in Figure 5, panel A top and bottom, discussed on page 53 of Schut et al (see, for example, page 36 line 10 to page 37 line 15, and page 53). Schut et al also teach that forward scatter light signal was measured and plotted versus fluorescence intensity for Cy5 labeled with YOPRO-1 (10x dilution) (see, for example, Figure 4 and page 52). Schut et al further teach that flow cytometric analysis was performed using a Becton Dickson FACScalibur equipped with a 15 mW argon laser (488 nm) and a 10 mW red diode laser (635 nm) (see, for example, page 32, lines 1-10 and page 34, lines 10-33).

Furthermore, Schut et al teach that the method described above can be used to quantitatively measure microorganisms present in a sample in addition to identifying the microorganisms present in the sample. In particular, Schut et al teach that the method can be used to quantitatively measure the flora present in human and animal fecal samples. Schut et al further teach that the method is useful for studying the effects of probiotics on intestinal flora in humans and animals, as well as to identify and quantify

the presence of microorganisms in wastewater treatment systems, in addition to several other beneficial uses (see, for example, page 19 lines 19-36, page 20, lines 1-15, and 34 through page 21).

Therefore, the reference is deemed to anticipate the instant claims above.

Response to Arguments

Applicant's arguments filed 10 June 2008 have been fully considered but they are not persuasive. Applicant argues that Schut et al fail to disclose or suggest the claimed invention, because Schut et al merely disclose a method which utilizes FISH and further, that Schut et al do not disclose how to use their enzyme treatments in any real complex microbial sample such as a fecal sample. The Examiner respectfully disagrees with Applicant's arguments.

Schut et al teach a method wherein samples containing more than one microorganism, such as those found in human and animal intestines, can be analyzed for the presence and quantity of microorganisms using fluorescent probes and flow cytometry. The utilization of FISH is only one of several methods disclosed by Schut et al. Furthermore, Applicant argues that Schut et al uses enzymes to allow probes to access nucleic acids in microorganisms, but since the instant claims use the language "comprising," there is nothing in the claims that would indicate that enzymes cannot be used in the present invention.

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies

(i.e., the use of complex microbial samples, such as fecal samples) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Maintained Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 37-53, 55-57, 68 and 71 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schut et al in view of Matsumoto et al (US 5,888,823).

Schut et al is relied upon for the reasons set forth above.

Schut et al do not expressly teach that microparticles are separated from the sample.

Matsumoto et al beneficially teach a standard fluid for flow cytometry which can be used in quality control and calibration of flow cytometers, and which comprises a fluid and particles which stain similarly to the cells being measured in the cytometer, such as bacteria. Matsumoto et al teach that the fluid allows for counting of cells of interest and does not interfere with fluorescence and scattered light intensities of stained cells. Furthermore, Matsumoto et al beneficially teach that the calibrating

particles of the fluid are measured by the flow cytometer using fluorescence and scattered light intensity (see, for example, Abstract and col. 3-6).

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the methods disclosed by Schut et al based upon the beneficial teachings provided by Matsumoto et al, with respect to the art-recognized method of providing a standard fluid containing particles for counting cells in a flow cytometer, as discussed above. Furthermore, Matsumoto et al beneficially teach that bacteria are among the types of cells which can be enumerated using the standard fluid demonstrated and that the fluorescence and light scattering of the cells are not disrupted by the standard, but that the standard can be used as a calibration tool, and therefore, it would have been both obvious and beneficial for the skilled artisan to use the methods taught by Schut et al in combination with the standard fluid of Matsumoto et al so as to provide an accurate method for identifying and enumerating bacteria in samples.

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole, was *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made, as evidenced by the cited references, especially in the absence of evidence to the contrary.

Claim 54 is rejected under 35 U.S.C. 103(a) as being unpatentable over Schut et al in view of Matsumato et al as applied to claims 37-53, 55-57 and 67-68 above, and further in view of Wallner et al (Cytometry 1993).

Schut et al and Matsumato et al are relied upon for the reasons set forth above,

Schut et al and Matsumato et al do not expressly teach a method wherein the at least two light sources are disposed at a distance from each other, signal delay equipment is used to delay measuring signals being created by means of the first and optionally the subsequent light sources.

Wallner et al beneficially teach a method similar to that of Schut et al, wherein bacteria in a sample are identified by fluorescent probes for 16S rRNA. Wallner et al teach a method using a FACStar Plus flow cytometer equipped with two argon lasers to measure forward angle light scatter, right angle light side scatter, and fluorescent intensity of the microbial cells. Wallner et al teach that during two color measurements, the emission light, excited sequentially by the two lasers, was split by the standard half mirror and fed to the respective photomultiplier tube (see, for example, Abstract and page 138).

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the methods disclosed by Schut et al based upon the beneficial teachings provided by Matsumoto et al, with respect to the art-recognized method of providing a standard fluid containing particles for counting cells in a flow cytometer, and by Wallner et al, with respect to the art-recognized method using two different light sources for flow cytometry, as discussed above. Furthermore, Wallner et

al beneficially teach that emission light is fed to the appropriate photomultiplier tube upon sequential excitation of the two light sources, and therefore, it would have been both obvious and beneficial for the skilled artisan to use the methods taught by Schut et al in combination with the method of Matsumato et al and Wallner et al so as to perform accurate measurements of the emission light from the sample.

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole, was *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made, as evidenced by the cited references, especially in the absence of evidence to the contrary.

Response to Arguments

Applicant's arguments filed 10 June 2008 have been fully considered but they are not persuasive. Applicant argues that Schut et al fail to disclose or suggest the claimed invention, because Schut et al merely disclose a method which utilizes FISH and further, that Schut et al do not disclose how to use their enzyme treatments in any real complex microbial sample such as a fecal sample. The Examiner respectfully disagrees with Applicant's arguments.

Schut et al teach a method wherein samples containing more than one microorganism, such as those found in human and animal intestines, can be analyzed for the presence and quantity of microorganisms using fluorescent probes and flow cytometry. The utilization of FISH is only one of several methods disclosed by Schut et

al. Furthermore, Applicant argues that Schut et al uses enzymes to allow probes to access nucleic acids in microorganisms, but since the instant claims use the language "comprising," there is nothing in the claims that would indicate that enzymes cannot be used in the present invention.

Applicant further argues that neither Matsumoto et al nor Wallner et al disclose or suggest the present invention as claimed, furthermore, do not render it obvious in view of Schut et al. Applicant further argues that the cited references do not solve several problems which are solved by the present invention, found on pages 23-24 of Applicant's arguments filed 10 June 2008. However, the problems identified by Applicant that are presumed to be solved by the instant invention, are not found in the claims as drafted (e.g., an absolute count of total/all microorganisms from a complex microbial sample, an absolute count of the probe defined target microorganisms from a complex microbial sample, an absolute count of "false target microorganisms," and how to analyze accurately all these parameter from a complex microbial sample in just a single analysis and single plot and to do so without the use of enzymes).

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., the use of complex microbial samples, such as fecal samples; the suggestion that the microparticles of claim 37 are several times bigger than bacteria and smaller than eukaryotic cells, and that they do not show the same fluorescence intensity and scatter light intensity; or that the analysis of samples containing numerous kinds of microorganisms by flow cytometry is done in one analysis to demonstrate target

microorganisms, other microorganisms, and other particles of a sample in one graph) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Conclusion

No claims allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications should be directed to examiner Amanda P. Wood whose telephone number is (571) 272-8141. The examiner can normally be reached on Mon-Fri 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jon Weber can be reached on (571) 272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

APW
Examiner
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